Indoor Population Structure of The Dry Rot Fungus, *Serpula lacrymans*

Master of Science Thesis

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ABSTRACT

The dry rot fungus *Serpula lacrymans* is known as one of the most aggressive fungal house invaders in temperate regions. Recent genetic and phylogeographic studies have shed light on the distribution, colonisation and population structure of *S. lacrymans* on a broader, global scale. Yet, even though *S. lacrymans* have been long known by both unfortunate house owners and researchers, little is still known about its population structure on a smaller scale, including within buildings. Therefore, a combination of vegetative compatibility tests and molecular techniques were used to investigate the indoor distribution of *S. lacrymans* in six houses in Norway. Vegetative compatibility experiments corroborated earlier findings that vegetative compatibility not necessarily corresponds to the same individual (genet) of *S. lacrymans*, as different genets often display the same VC group affiliation. The results also indicate that infections in Norway often are made up of a single genet with the ability to colonise an entire house. Interestingly, the molecular investigations indicated that some of the genets stem from parents with identical mating genotype. However, this is probably more likely a result of low levels of molecular variation in the analysed markers. I also speculate that this observation may be caused by same-sex mating in *S. lacrymans*, a recently discovered form of homothallism in fungi. The presence of a formerly unrecognised MAT B allele is also indicated by the molecular data.

INTRODUCTION

The dry rot fungus *Serpula lacrymans* is known as one of the most aggressive fungal house invaders in temperate regions. The species decompose wood in houses through brown rot decay, and causes large economic losses every year.

Several features contribute to *Serpula lacrymans* being regarded as one of the most devastating fungal invaders of houses. The fungus can remain dormant in buildings for long periods of time, until the conditions are suitable. Optimal conditions include a temperature at about 23°C and moisture levels at about 20-55%, as well as a source of calcium or iron, which is needed for breaking down cellulose (Mattsson, 2010, Jennings and Bravery, 1991). *S. lacrymans* gains nutrition through brown rot, a process where the cellulose and hemicellulose parts of the wood are degraded and utilized by the fungus, whereas the lignin remains (Schmidt, 2006, Watkinson and Eastwood, 2012). The wood becomes characteristically brown during this process. It shrinks and breaks into cubicles, and is no longer able to support any weight.

Once established, the fungus shows a remarkable ability to spread further into the building. The genus name *Serpula* means "little snake" in Latin, and most likely points to the fungus' ability to form rhizomorphs, cable like clusters of hyphae utilized in the transport of water and nutrients (Fig. 1d). These structures likely promote local vegetative spread of the fungus, as they can grow to be of considerable length and thickness, as well as grow on a variety of substrates (Jennings and Bravery, 1991, Mattsson, 2010). The species epithet *lacrymans* translates as "shedding tears" in Latin, reflecting the fungus' ability to "cry" droplets of water under certain conditions (Jennings and Bravery, 1991). Such droplets promote the upkeep of favourable growth conditions by ensuring a continued moist environment (Fig. 1c).

Serpula lacrymans displays a typical basidiomycete life cycle (Brown and Casselton, 2001, Heitman et al., 2007). The cycle starts with the formation of primary mycelium from haploid sexual spores. This mycelium is comprised of monokaryotic hyphae, i.e. mycelium with one nucleus per hyphal cell. This haploid phase is thought to be short lived, as it is rarely observed in nature. At the next step of the cycle, different primary mycelia meet. They fuse, and mating type determination takes place. If the two nuclei have different alleles present at each of the mating type loci,

mating takes place, which results in plasmogamy and the formation of a dikaryotic mycelium. Since the basidiomycete mycelium includes two separate nuclei, mating does not necessarily need to include two monokaryotic mycelia, meaning that mating also happens between monokaryotic and dikaryotic hyphae. Moreover, spores may have the ability to dikaryotise the primary mycelium (Anderson and Kohn, 2007). The dikaryotic mycelium is comprised of hyphae with two distinct nuclei present in each hyphal cell, one from each parent. The dikaryotic phase is the predominant stage of the basidiomycete life cycle. After mycelial growth the resupinate, pancake-like basidiocarps are formed. The size of the basidiocarps can vary from a few centimeters in diameter, up to several decimetres (Mattsson, 2010, Jennings and Bravery, 1991) (Fig. 1c). Karyogami, the fusion of nuclei, will only take place after fruiting is initiated. Meiosis is then induced, followed by dispersal of new haploid spores, after which the cycle is repeated. A single fruiting body is capable of producing billions of basidiospores (Schmidt, 2006, Jennings and Bravery, 1991). These are characteristic rusty brown and can cover large extents of an infected house (Fig. 1a and b).

Systematically, *S. lacrymans* belongs to the family Serpulaceae, in the order Boletales (Skrede et al., 2011, Watkinson and Eastwood, 2012, Binder and Hibbett, 2006). Serpulaceae consists of other *Serpula* species and the ectomychorrizal genera *Austropaxillus* and *Gymnopaxillus* (Binder and Hibbett, 2006, Skrede et al., 2011).

The species *S. lacrymans* comprises two main lineages that form well-differentiated cryptic species (Kauserud et al., 2007, Kauserud et al., 2012). *Serpula lacrymans* var. *shastensis* resides mainly in North America, and is often denoted the "wild" form of *S. lacrymans*, as it has only been found in natural habitats. *Serpula lacrymans* var. *lacrymans* can occur both in nature and in buildings, although it is far more commonly found inside buildings (Palfreyman et al., 1995, Jennings and Bravery, 1991).

Population genetic studies have shown that *var. lacrymans* most likely originated in mainland Asia, from where it has recently expanded to other temperate regions worldwide (Kauserud et al., 2007). Genetic analyses indicate long distance dispersal from Asia to Europe, and further to Oceania and North America, followed by local population expansions. The long distance dispersal was probably mediated by human activities such as transportation of infected wood materials (Watkinson and Eastwood, 2012, Kauserud et al., 2007). A population of *var*. *lacrymans* in Japan is a result of a separate colonisation from mainland Asia. The Japanese population of *var.*

lacrymans has higher genetic variation than what appears in the European population, (Kauserud et al., 2007, Kauserud et al., 2004, Engh et al., 2010a, Kauserud et al., 2012), which suggests that the European population has experienced a tighter bottleneck prior to expansion (Kauserud et al., 2004).

In agreement with the low genetic variation, a low number of vegetative compatibility types (6) have been found in Europe (Kauserud et al., 2006). Vegetative compatibility governs the fusion of secondary mycelium. Fusion of secondary mycelia will only take place if there is a genotypic similarity between the alleles present at the vegetative compatibility loci, denoted *vic*. This reduces the flow of nutrients and sharing of habitat between competitive individuals. In addition, the vegetative compatibility principle is thought to provide a protective mechanism against transmission of infectious cytoplasmic elements such as mycoviruses (Malik and Vilgalys, 1999, Worrall, 1999, Milgroom, 1999).

The mating type diversity in the European var. *lacrymans* has also been shown to be relatively low compared to that of other fungal species (Kauserud et al., 2004, Kauserud et al., 2006). Mating types denote the sexual identities of fungi. For sexual reproduction to take place, two individuals need to be of different mating types, a principle known as mating compatibility (Brown and Casselton, 2001, Heitman et al., 2007). The mating type is determined by the alleles present at the mating type (MAT) loci, and two individuals are compatible if these alleles differ from each other. Basidiomycetes are unique in having species with tetrapolar mating systems, i.e. two mating type loci (Heitman et al., 2007). These loci are often denoted MAT A and MAT B. The MAT A locus encodes homeodomain transcription factors, whereas MAT B encodes pheromones and pheromone receptors (Brown and Casselton, 2001, Heitman et al., 2007). In addition to mate recognition and regulation, these loci are also involved in processes such as clamp formation, migration and pairing of nuclei in the formation of a dikaryotic mycelium. The mating type loci are composed of multiple, tightly linked and redundant subloci. Recombination between these subloci can result in generation of a huge number of mating types. This serves as an explanation to the presence of up to several hundreds or even thousands of different mating type alleles in some basidiomycetes (Brown and Casselton, 2001, Heitman et al., 2007). The exact number of mating types present in European var. *lacrymans* has yet to be determined. In a study by Kauserud et al. (2006) based on mating experiments, four (MAT A) and five (MAT B) mating types were detected,

respectively. Using genome sequence data, Skrede et al. (2013) were able to annotate the two mating type loci of *S. lacrymans*. Markers linked to the MAT A and MAT B regions were developed and used to investigate the mating type diversity of the European population, which resulted in the recognition of at least three MAT A alleles and four MAT B alleles. Two of the functional mating types found in Kauserud et al. (2006) were not detected in the study by Skrede et al. (2013). This is not surprising, as the MAT linked markers functions as proxies for the whole MAT gene complexes, meaning that genotypic variation could be masked due to low resolution of the selected markers (Skrede et al., 2013).

Although recent genetic and phylogenetic studies have shed light on the distribution, colonisation and population structure of *S. lacrymans* on a global and European scale, little is known about its population structure on a smaller scale, including within buildings. My study therefore seeks to map the distribution of *S. lacrymans var. lacrymans* within buildings using molecular techniques and vegetative compatibility-tests, with emphasis on the following questions:

- Do we typically find one or more genetic individuals (genets) of *S. lacrymans* in colonised buildings?
- If there is more than one genet present in a building: How are the different genets distributed on a spatial scale? Do they share the same living space, or do they occupy different "zones" of a building?
- Are the genets within the same building genetically more similar compared to across buildings? This could then indicate non-random mating.

a

b

c

d

e

Fig. 1a and b: Heavy infections of *S. lacrymans* can cause building collapse due to extensive brown rot. Fruiting bodies produce billions of spores, often seen as a thin layer of rust coloured “dust” covering all exposed surfaces. **c** Fruiting bodies can grow to become of considerable size. **d** *S. lacrymans* produces rhizomorphs that can grow both thick and long, even over inorganic substrates. **e** *S. lacrymans* is also known for its droplet formation, here seen in an interaction zone between two laboratory cultures. All photographs are courtesy of the author.

MATERIALS AND METHODS

Sample collection

A total of 38 samples, collected from six buildings, are included in the study. These are listed in Table 1. The samples consist mainly of fruiting bodies, with the exception of two isolates that were collected before the project started. Collection was done in all rooms where fruiting bodies were present in each building. Parts of the fruiting bodies were carefully loosened from their substrate and put into plastic bags using gloves. A sketch of the location was drawn when possible.

Culturing

Cultures were obtained from the fruiting bodies. All handling of cultures were done in biological safety cabinets using sterile scalpels to avoid contamination. Small pieces of material (about 2-5 mm^3 in size) were placed on 9 cm Petri dishes containing 2 % malt extract agar (MEA). The dishes were sealed with parafilm and stored in incubators at 19 ºC. When the mycelium started to grow, small pieces were transferred to new Petri dishes with 2 % MEA in order to obtain axenic cultures. Remaining parts of the fruiting bodies were dried in and kept in sealed paper bags in case some samples failed to grow in culture.

Vegetative compatibility

Vegetative compatibility tests (VC tests) have been extensively used to investigate population biology and delimit genets (genetically distinct individuals) of basidiomycetes. Such tests provide a relatively easy way of assessing whether cultures belong to the same vegetative compatibility group or not, i.e. whether or not the cultures recognise the other culture as itself (Malik and Vilgalys, 1999, Kauserud et al., 2006). In my study, VC tests were conducted between the cultures indicated in Table 2, during February to April 2012. Mycelium from two samples were placed approximately 2 cm apart on 9 cm Petri dishes containing 2 % MEA. The Petri dishes where then incubated at 19 ºC for six weeks, after which vegetative incompatibility was assessed morphologically. Two cultures were scored as vegetative incompatible when a demarcation zone was formed between them, seen as a canyon-like barrier between the mycelia stemming from the different cultures (Fig. 2a). Some samples

would not produce axenic cultures, were collected at a later stage or were only available as DNA isolates, and therefore could not be included in the VC tests.

DNA extraction and isolation

DNA was extracted from all the axenic cultures. In addition, DNA was extracted from some dried specimens in order to increase the sample size (Table 1). Due to contamination, two of the cultures included in the confrontation experiment were lost before DNA extraction took place, and therefore are absent from the remaining analyses.

DNA was extracted with a cetyltrimethylammonium bromide (CTAB) and chloroform protocol as described by Murray and Thompson (1980). Small pieces of fungal material (approximately 2 mm^3) were deposited in 2 ml Eppendorf tubes containing CTAB, mercaptoethanol and tungsten beads, and then crushed in a MM301 Mixer Mill (Retsch GmbH & Co., Haan, Germany). The standard protocol was modified by removing the mercaptoethanol step for samples extracted late in the project, following Tung Nguyen et al. (2009), as specified in Supplementary Table 1. The final DNA was dissolved in 60 µl milli-Q H_2O and stored in a freezer at -20 °C.

Primers, polymerase chain reaction (PCR) and sequencing

For assessment of mating types, two newly developed primer pairs for the the MAT A and MAT B regions of *S. lacrymans* were used (Skrede et al., 2013). These markers amplify small regions of the larger mating type loci. The marker for the MAT A region is located in the 3'UTR of the MAT A homeodomain II (HD2) transcription factor (MATA 11F 5'-GCC TCT TGG TTG TTT TTA TTG 3' and MATA 7R 5'-GCT GTG AGT GCT AGT GCT ACA-3'). The marker for the MAT B region is located in the pheromone receptor gene 4 (Slrcb4; MATB 1F - 5'-TCC TTC GCA CCT CAT GGC AGC-3' and MATB 1R - 5'-TCG TAG GAC GGC ATC CAA AGC-3') (Skrede et al., 2013)).

PuReTaq Ready-To-Go™ PCR Beads (GE healthcare, Waukesha, WI) were used for most PCR reactions. The protocol for PCR Beads includes a bead provided by the manufacturer, which contains enzyme and buffer, and the addition of 1 µl of each primer, 21 µl milliQ H_2O and 2 µl DNA. For the eight first MAT A reactions (Odin 6, 7, 7b and 8, Skåbu 1, 2, 3 and 7) a protocol using the enzyme Dynazyme

EXT DNA polymerase (ThermoFisher Scientific, Waltham, MA) were used in 25 µl reactions. The protocol for Dynazyme EXT includes 0.2 µl enzyme and 2.5 µl buffer provided by the manufacturer, 2.5 µl dNTPs of 2 mM concentration, 1.5 µl of each primer at a 5 mM concentration, 14.8 µl milliQ H_2O and 2 µl DNA.

The PCR was conducted on the Mastercycler thermocycler (Eppendorf, Hamburg, Germany) according to the following protocols: Initial denaturation at 94 °C for 2 minutes was followed by 30 cycles of denaturation at 94 °C for 30 seconds, 45 seconds of annealing at 57 °C (MAT A)/ 52 °C (MAT B) and 1 minute extension at 72 °C, with a final extension of 7 minutes at 72 °C before an indefinite hold at 16 °C.

PCR products were purified using 2 µl 10 times diluted ExoStar (GE healthcare, Waukesha, WI) per 5 µl PCR products. This protocol included incubation at 37 °C for 30 minutes, followed by 15 minutes at 80 °C, and a final indefinite hold at 10 °C on the Eppendorf thermocycler. Sanger sequencing analyses were done by the ABI-lab at CEES, University of Oslo, on a 3730 XL DNA analyzer (Life Technologies, Foster City, CA).

Sequence analyses

BioEdit 7 (Hall, 1999) was used for assessing sequence quality. All chromatograms were inspected manually and all polymorphisms were verified. The sequences were manually aligned and assigned to parental mating type alleles using information from Skrede et al. (2013). In Skrede et al. (2013) MAT alleles were obtained by cloning the PCR products of dikaryons or direct sequencing of monokaryons. This resulted in haplophase sequence data. The combination of polymorphisms in the ‘dikaryophase’ data obtained in my study were compared to the sequences from Skrede et al. (2013), to infer the most likely combination of haplophase sequences.

Table 1: Sample overview

All samples were collected fresh, except for the samples marked SL, which were collected before the project was started.

Sample ID	Location	Date	Type of material	DNA extract	Collector(s)
Odin 6	Odins gate 4, Oslo, Norway	21.09.2011	Fruiting body	Culture	Mycoteam AS, author
Odin 7	Odins gate 4, Oslo, Norway	21.09.2011	Fruiting body	Culture	Mycoteam AS, author
Odin 7b	Odins gate 4, Oslo, Norway	21.09.2011	Fruiting body	Culture	Mycoteam AS, author
Odin 8	Odins gate 4, Oslo, Norway	21.09.2011	Fruiting body	Culture	Mycoteam AS, author
Skåbu 1	Gudbrandsdalen, Norway	03.10.2011	Fruiting body	Culture	Mycoteam AS
Skåbu 2	Gudbrandsdalen, Norway	03.10.2011	Fruiting body	Culture	Mycoteam AS
Skåbu 3	Gudbrandsdalen, Norway	03.10.2011	Fruiting body	Culture	Mycoteam AS
Skåbu 5	Gudbrandsdalen, Norway	03.10.2011	Fruiting body	Culture	Mycoteam AS
Skåbu 7	Gudbrandsdalen, Norway	03.10.2011	Fruiting body	Culture	Mycoteam AS
SL 557	Østbanehallen, Oslo, Norway	04.04.2011	Mycelium	Mycelium	Mycoteam AS, Inger Skrede
SL 558	Østbanehallen, Oslo, Norway	04.04.2011	Mycelium	Mycelium	Mycoteam AS, Inger Skrede
Therese 2	Thereses gate 6, Oslo, Norway	01.10.2011	Fruiting body	Culture	Mycoteam AS, Håvard Kauserud
Therese 3	Thereses gate 6, Oslo, Norway	01.10.2011	Fruiting body	Culture	Mycoteam AS, Håvard Kauserud
Therese 4	Thereses gate 6, Oslo, Norway	01.10.2011	Fruiting body	Culture	Mycoteam AS, Håvard Kauserud
Therese 5	Thereses gate 6, Oslo, Norway	01.10.2011	Fruiting body	Culture	Mycoteam AS, Håvard Kauserud
Therese T1	Thereses gate 6, Oslo, Norway	01.10.2011	Fruiting body	Dried fruiting body	Mycoteam AS, Håvard Kauserud
Therese T2	Thereses gate 6, Oslo, Norway	01.10.2011	Fruiting body	Dried fruiting body	Mycoteam AS, Håvard Kauserud
Therese T3	Thereses gate 6, Oslo, Norway	01.10.2011	Fruiting body	Dried fruiting body	Mycoteam AS, Håvard Kauserud

Therese T4	Thereses gate 6, Oslo, Norway	01.10.2011	Fruiting body	Dried fruiting body	Mycoteam AS, Håvard Kauserud
Therese T5	Thereses gate 6, Oslo, Norway	01.10.2011	Fruiting body	Dried fruiting body	Mycoteam AS, Håvard Kauserud
Therese T6	Thereses gate 6, Oslo, Norway	01.10.2011	Fruiting body	Dried fruiting body	Mycoteam AS, Håvard Kauserud
Therese T7	Thereses gate 6, Oslo, Norway	01.10.2011	Fruiting body	Dried fruiting body	Mycoteam AS, Håvard Kauserud
Vahl 1	Vahl Skole, Oslo, Norway	01.09.2012	Fruiting body	Culture	Mycoteam AS
Vahl 2	Vahl Skole, Oslo, Norway	01.09.2012	Fruiting body	Culture	Mycoteam AS
Vahl 3	Vahl Skole, Oslo, Norway	01.09.2012	Fruiting body	Culture	Mycoteam AS
Vahl 8	Vahl Skole, Oslo, Norway	01.09.2012	Fruiting body	Culture	Mycoteam AS
Ås 1	Campus, UMB, Ås, Norway	26.03.2012	Fruiting body	Dried fruiting body	Håvard Kauserud, author
Ås 2	Campus, UMB, Ås, Norway	26.03.2012	Fruiting body	Dried fruiting body	Håvard Kauserud, author
Ås 3	Campus, UMB, Ås, Norway	26.03.2012	Fruiting body	Culture	Håvard Kauserud, author
Ås 5	Campus, UMB, Ås, Norway	26.03.2012	Fruiting body	Dried fruiting body	Håvard Kauserud, author
Ås 8	Campus, UMB, Ås, Norway	26.03.2012	Fruiting body	Culture	Håvard Kauserud, author
Ås 9	Campus, UMB, Ås, Norway	26.03.2012	Fruiting body	Dried fruiting body	Håvard Kauserud, author
Ås 10	Campus, UMB, Ås, Norway	26.03.2012	Fruiting body	Dried fruiting body	Håvard Kauserud, author
Ås 12	Campus, UMB, Ås, Norway	26.03.2012	Fruiting body	Dried fruiting body	Håvard Kauserud, author
Ås 13	Campus, UMB, Ås, Norway	26.03.2012	Fruiting body	Dried fruiting body	Håvard Kauserud, author
Ås 14	Campus, UMB, Ås, Norway	26.03.2012	Fruiting body	Dried fruiting body	Håvard Kauserud, author
Ås 15	Campus, UMB, Ås, Norway	26.03.2012	Fruiting body	Dried fruiting body	Håvard Kauserud, author
Ås 16	Campus, UMB, Ås, Norway	26.03.2012	Fruiting body	Dried fruiting body	Håvard Kauserud, author

RESULTS

Vegetative compatibility tests

S.lacrymans is known to form clearly distinguishable barriers, often denoted barrages, when two cultures belonging to different VC groups meet in a Petri dish (Fig. 2a). By manually inspecting the Petri dishes, the VC tests revealed two VC groups (Table 2). A barrage was seen in 40 of the 169 VC tests. No barrages (Fig. 2b) were detected between cultures stemming from the same sample (controls) or from the same location.

Sequence data, distribution of MAT alleles

In total, 30 MAT A sequences and 27 MAT B sequences were obtained from the 38 samples included in this study (Table S2 in the supplementary material). Most of the sequences possessed a number of heterozygous positions with two nucleotides present at the same site. These polymorphisms made it possible to assign putative mating type alleles to the sequences, based on comparisons with the mating type alleles described in Skrede et al. (2013) (Table 3).

The sequences obtained in this study showed a fairly uniform distribution of mating types within buildings. No differences could be detected in the MAT A sequences between samples stemming from the same building. The MAT B sequences showed the same pattern, except for in one building (Odins gate, denoted Odin), where two genotypes were identified (Table 3b). The isolates Odin 6, 7 and 8 shared the same MAT B alleles, while Odin 7b seems to be the result of a crossing between parents with the B4 allele (Table 3a and b).

All previously identified MAT A (A1, A2 and A3) and MAT B alleles (B1, B2, B3 and B4) were recognised among the obtained sequences. However, a number of unexpected polymorphisms were found in the MAT B sequences of Odin 6, 7 and 8, which suggests the presence of an allele not previously described (Table 4). Comparisons towards the MAT B sequences from Skrede et al. (2013) indicate that the isolates Odin 6, 7 and 8 includes a B4 allele along with another allele, tentatively named b5. Note also that the Odin 7b isolate includes two identical (‘homozygous’) MAT B markers and that the Therese-samples display homozygosity on both loci, indicating parents with identical genotypes for both the MAT A and MAT B markers.

Sampling area

Fig. 3 and 4 provide sketches of two of the locations, giving information about the spatial distribution of the fruiting bodies included in my study. At UMB, Ås (Fig. 4), spores were found on most surfaces, and *S. lacrymans* had spread extensively throughout the building.

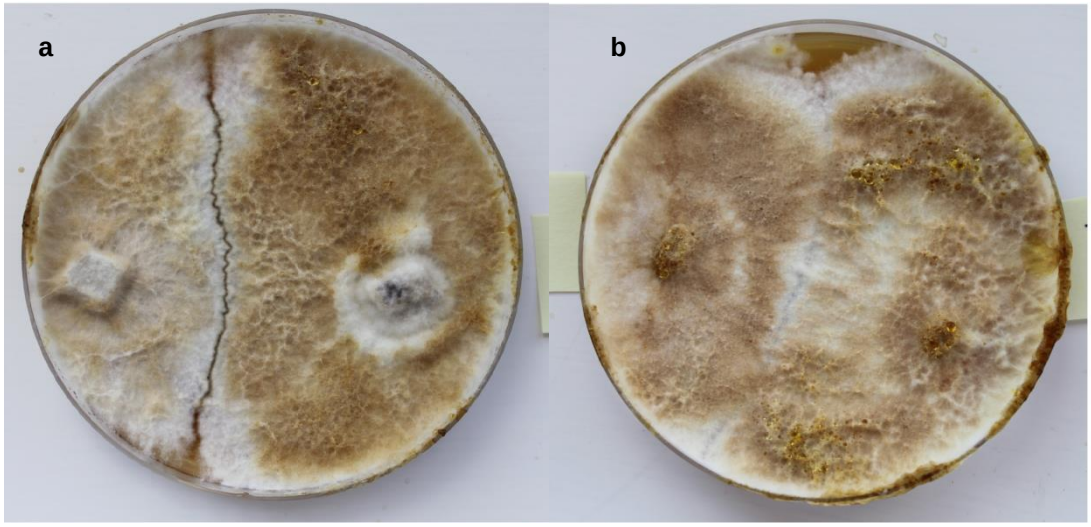

Fig. 2: Vegetative compatibility tests. **a** A distinct barrier indicates vegetative incompatibility. **b** Mycelia grow in a seemingly continuous fashion with no sharp barrier, indicating vegetative compatibility.

Table 2: Results from VC tests
The letter B indicates the formation of a barrage in the contact zone between the two mycelia in the Petri dish (Fig. 2a). The letter C (compatible) indicates that the mycelia grew in a seemingly continuous fashion with no visible border between them (Fig. 2b).

	Odin 6	Odin 7	Odin 7b	Odin 8	Therese 2	Therese 3	Therese 4	Therese 5	Skåbu 1	Skåbu 2	Skåbu 4	Skåbu 5	Skåbu 7
Odin 6	C	C	C	C	C	C	C	C	**B**	**B**	**B**	**B**	**B**
Odin 7		C	C	C	C	C	C	C	**B**	**B**	**B**	**B**	**B**
Odin 7b			C	C	C	C	C	C	**B**	**B**	**B**	**B**	**B**
Odin 8				C	C	C	C	C	**B**	**B**	**B**	**B**	**B**
Therese 2					C	C	C	C	**B**	**B**	**B**	**B**	**B**
Therese 3						C	C	C	**B**	**B**	**B**	**B**	**B**
Therese 4							C	C	**B**	**B**	**B**	**B**	**B**
Therese 5								C	**B**	**B**	**B**	**B**	**B**
Skåbu 1									C	C	C	C	C
Skåbu 2										C	C	C	C
Skåbu 4											C	C	C
Skåbu 5												C	C
Skåbu 7													C

Table 3a: Overview of polymorphic sites

Table showing bases present at polymorphic sites. Base abbreviations follow standard IUPAC nucleotide code with Y, R, K, M, W and S denoting polymorphisms. Y = C/T, R = A/G, K = A/T, M = A/C, W = A/T, S = G/C. A dash (-) indicates a gap, while a question mark (?) indicates that any base or a gap may be present at this site. To view the full sequences, see the alignments in Fig. S1 and S2 (supplementary).

	MAT A			MAT B															
Base position	83	95	116	14	48	77	78	79	95	184	247	250	329	330	348	353	364	380	433
MAT A1	T	A	T																
MAT A2	C	A	T																
MAT A3	C	G	G																
MAT B1				C	C	G	A	A	C	C	A	A	T	T	C	A	T	C	T
MAT B2				C	T	A	G	T	T	C	G	C	-	-	G	A	C	T	C
MAT B3				T	C	A	G	T	C	C	G	C	-	-	G	G	C	T	T
MAT B4				T	C	A	G	T	C	T	G	C	-	-	G	A	C	T	T
Therese	T	A	T	C	C	G	A	A	C	C	A	A	T	T	C	A	T	C	T
Ås	Y	R	K	Y	Y	A	G	T	Y	C	G	C	-	-	G	R	C	T	Y
SL	Y	R	K	Y	Y	A	G	T	Y	Y	G	C	-	-	G	A	C	T	Y
Vahl	C	R	K	T	C	A	G	T	C	Y	G	C	-	-	G	R	C	T	T
Skåbu	Y	R	K	Y	C	R	R	W	C	Y	R	M	Y	T	S	A	Y	Y	T
Odin	Y	R	K	Y	C	A	R	T	C	Y	R	C	Y	T	?	?	C	Y	Y
Odin7b	Y	R	K	T	C	A	G	T	C	T	G	C	-	-	G	A	C	T	T

Table 3b: Mating types found at each location

Summary of mating types deduced from the polymorphic sites for the different locations. Two MAT genotypes were present among the Odin-samples. For Odin 6, 7, and 8, it was only possible to find correspondence with one of the template MAT B alleles (see Table 4).

Sample ID	Mating type
Therese	A1A1, B1B1
Ås	A1A3, B2B3
SL	A1A3, B2B4
Vahl	A2A3, B3B4
Skåbu	A1A3, B1B4
Odin	A1A3, B4b5
Odin 7b	A1A3, B4B4

Table 4: Polymorphisms in the MAT B sequences of Odin 6, 7 and 8, and an outline of the b5 allele

The table gives an overview of all polymorphic sites detected in the MAT B sequences of Odin 6, 7 and 8. The polymorphic sites were compared to the B4 allele, which resulted in a possible outline of an unknown allele denoted b5. Gray shading indicates already known polymorphic sites. Due to the presence of many polymorphisms/possible gaps in the Odin-sequences, it was difficult to get meaningful reads from position 324-352 in the alignment. These positions were therefore excluded from the outline. Base abbreviations follow standard IUPAC nucleotide code with Y, R, K, M, W and S denoting polymorphisms. Y = C/T, R = A/G, K = A/T, M = A/C, W = A/T, S = G/C. A question mark (?) indicates that any base or a gap may be present at this site.

Base position	14	24	25	39	43	48	51	61	74	77	78	79	95	98	99
Odin 6, 7, 8	Y	R	R	R	S	C	Y	Y	Y	A	R	T	C	R	M
MAT b5	C	G	G	A	G	C	T	T	T	A	A	T	C	G	A
MAT B4	T	A	A	G	C	C	C	C	C	A	G	T	C	A	C

Base position	131	142	184	247	250	253	265	278	286	291	313	353	354	358	364
Odin 6, 7, 8	R	S	Y	R	C	Y	Y	R	Y	Y	Y	W	R	W	C
MAT b5	G	G	C	A	C	T	C	G	T	C	T	T	G	T	C
MAT B4	A	C	T	G	C	C	T	A	C	T	C	A	A	A	C

Base position	365	380	406	409	412	419	433	467	508	529	551	556	563	583	590
Odin 6, 7, 8	R	T	R	R	K	Y	Y	K	Y	R	S	M	R	R	R
MAT b5	A	T	A	A	G	T	C	T	T	G	C	C	G	A	G
MAT B4	G	T	G	G	T	C	T	G	C	A	G	A	A	G	A

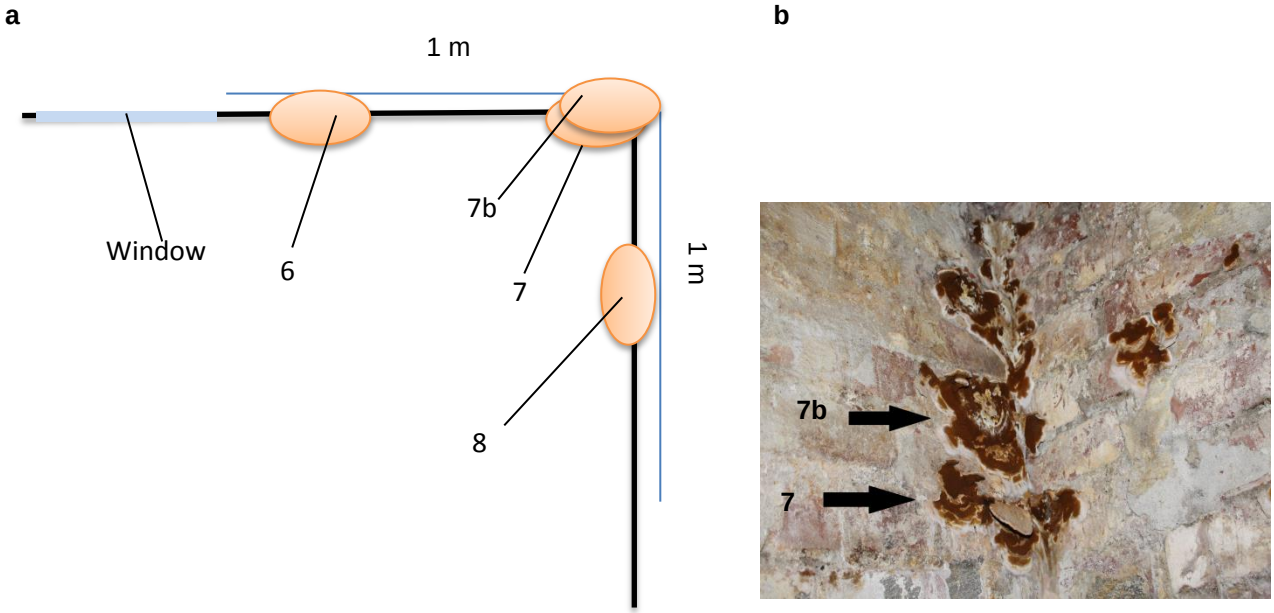

Fig. 3a Sketch of Odins gate 6. Fruiting bodies had erupted in one corner of the cellar. Numbers reflect sample IDs. It was difficult to determine whether the 7b sample was part of one bigger fruiting body or represented a distinct fruiting body growing in close proximity to the sample named Odin 7. **b** is a photo of the location. Odin 7 and 7b are indicated by arrows (photo by author). (See Fig. S 3 in the supplementary material for higher resolution of photography).

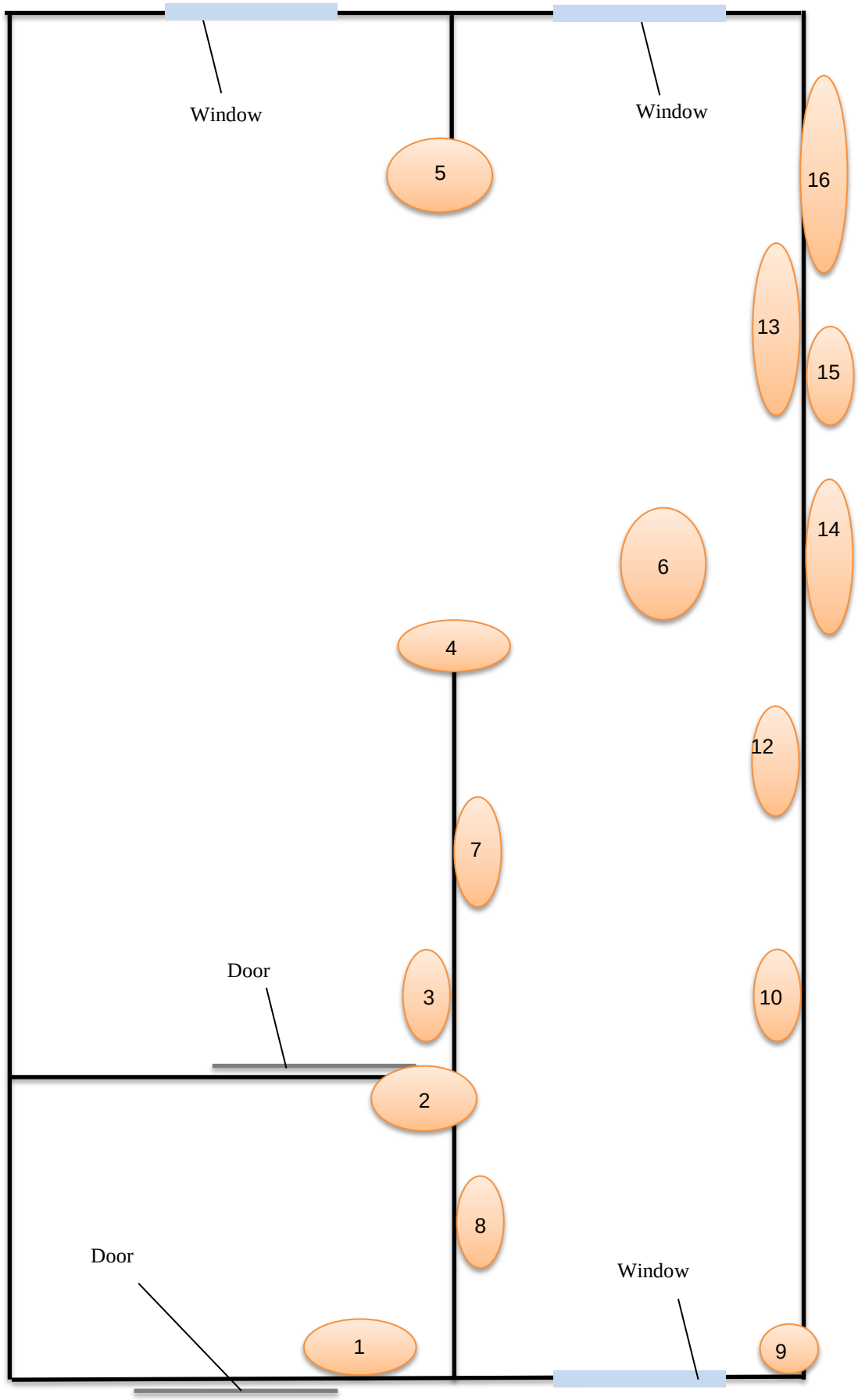

Fig. 4 Sketch of the location at UMB, Ås. Note that three fruiting bodies were collected from the building exterior.

DISCUSSION

Vegetative compatibility

Vegetative compatibility (VC) refers to the ability of fungi to separate self from nonself. The VC response is governed by genes at the *vic* loci, and the number of *vic* loci seems to vary extensively between species. For instance, it has been postulated that the ascomycete *Podospora anserina* harbours at least 17 such loci (Glass and Kuldau, 1992), while three to four *vic* loci are probably present in the basidiomycete *Heterobasidium annosum* (Hansen et al., 1993). For *S. lacrymans*, previous studies have suggested at least two polymorphic bi-allelic *vic* loci and six VC types in the European population (Kauserud et al. 2006). Vegetative compatibility tests (VC tests) have been extensively used to discriminate between individual genets of conspecific fungi (Malik and Vilgalys, 1999, Hansen and Hamelin, 1999). Individuals belong to the same VC type if they share an identical set of *vic* alleles. Fusion of secondary mycelia to form a larger, physiologically connected organism usually only takes place when mycelia share similar *vic* genotypes, i.e. they recognise each other as "self". Vegetative confrontations between genotypes recognising each other as nonself are usually followed by a rejection response that allows the persistence of each mycelium as a discrete genetic and physiological unit (Malik and Vilgalys, 1999). The rejection response varies with species, and in *S. lacrymans*, it is seen as a formation of a clear barrage zone between to mycelia of different *vic* genotypes (Fig. 2a).

In basidiomycetes in general, naturally occurring dikaryotic isolates tend to be incompatible when paired, suggesting that VC types normally correspond to genetic individuals. This is supported by a number of studies, where molecular markers or mating type markers tends to correspond fairly well to those defined by VC (Malik and Vilgalys, 1999, Dahlberg and Stenlid, 1994). However, no strong link between genetic uniqueness and VC type has been found in the European population of *S. lacrymans* (Kauserud et al., 2006). In fact, the fungus has been shown to partly lack the ability to recognise self from nonself, allowing fusion between mycelia of different genotypes (Kauserud et al., 2004, Kauserud, 2004, Kauserud et al., 2006). This is corroborated by the results in my study (Table 2) where only two VC types were found. Isolates from Skåbu (Gudbrandsdalen) showed a clear distinction from the samples from both Odins gate and Thereses gate (Oslo), while the samples from Oslo seems vegetative compatible to each other, overall indicating the presence of only two VC types.

However, the molecular MAT markers identified the samples from Thereses gate as having a different MAT genotype than the samples from Odins gate, and moreover, Odins gate harbours at least two genets as judged by mating types (Table 3b). In other words, the molecular markers distinguish between at least four genets, while only two VC types were detected in the VC tests, supporting the earlier findings that there is a poor link between VC types and genets in *S. lacrymans*.

Vegetative compatibility is thought to provide a protective mechanism against transmission of harmful cytoplasmic elements between mycelia. Moreover, the prevention of fusion between two genets may ensure that potentially costly genomic conflicts are avoided (Worrall, 1999, Malik and Vilgalys, 1999, Milgroom, 1999). VC is probably also linked to the competition for resources. Fungi use their substrate directly as a source of food, and space is therefore a potentially limited resource that might greatly influence the resources available to an organism, the size it is able to attain, and ultimately its fitness (Malik and Vilgalys, 1999).

One likely explanation for the low allelic diversity in the European population of *S. lacrymans,* relates to the observations that it has a narrow genetic base in Europe compared to e.g. the closely related species *S. himantoides* (Kauserud et al., 2004, Kauserud et al., 2006, Engh et al., 2010a). The poor ability to recognise self from nonself is most likely the result of a recent genetic bottleneck event that caused the introduction of only a few genets. Correspondingly, only a few *vic* alleles were introduced, resulting in several genets sharing the same *vic* alleles. One may speculate that the proposed low number of genets present in the same environment, taken together with the aggressive nature of the fungus, as well as the observation that the fungus generally have few competitors in its surroundings, may constitute features that lower the negative effect of the few *vic* alleles.

Distribution of MAT alleles

For mating to occur in heterothallic tetrapolar fungi like *S. lacrymans*, the presence of different alleles at the mating type loci, MAT A and MAT B, is required. Hence, it is expected to find polymorphisms and heterozygous positions in the obtained "composite" sequences representing the different MAT A and MAT B alleles. However, several of the obtained sequences did not display the presumed levels of polymorphisms. These sequences could only be traced to one possible parental MAT genotype. It must be noted that both MAT markers used in this study only function as proxies for the whole mating

type complex since the markers only covers a small region of the MAT gene complex. Therefore, some molecular variation is likely not to be reflected due to the limited resolution of the selected markers, as was also postulated in the study by Skrede et al. (2013). In their study two previously identified functional mating types (Kauserud et al., 2006, Schmidt and Moreth-Kebernik, 1991) were not recognised, probably due to lack of resolution in the developed markers.

Another intriguing possibility, relates to the recent discovery of a novel form of homothallism in fungi, where isolates of a single mating type are capable of mating with either themselves or other members of the population of the same mating type (Lin et al., 2005, Kües et al., 2011). This kind of same-sex mating was discovered in the human pathogenic basidiomycete *Cryptococchus neoformans*, and population genetics studies have shown that this unisexual mode of reproduction occurs in nature. Same-sex mating also occurs naturally in the closely related *C. neoformans var. grubii* and *C. gattii* (Kües et al., 2011). Other examples include the human pathogenic ascomycete *Candida albicans*, which was recently discovered to undergo same-sex mating (Kües et al., 2011). It is unclear whether *S. lacrymans* also display this kind of reproduction, but the genotype distributions revealed by population genetic analyses indicate that outcrossing dominates (Engh et al., 2010b).

The presence of a "new" allele was discovered in three of the samples of my study, which was tentatively called b5. Previous studies based on mating experiments have indicated the presence of at least four functional MAT A alleles and five functional MAT B alleles in the European population of *S. lacrymans* (Kauserud et al., 2006), but molecular investigations of mating type diversity have so far only tentatively identified three and four of the respective mating types (Skrede et al., 2013). The new b5 allele found here probably represent a sixth mating type in the European population as two functional mating types in Skrede et al. (2013) had the MAT B3 allele. However, replication and more extensive studies (including cloning) are necessary to investigate the full diversity of MAT alleles in Europe.

Population structure of *Serpula lacrymans* within buildings

The data in this thesis indicate that *S. lacrymans* infections in buildings in Norway often are made up of a single genet, as more than one multi-locus genotype was found in only one location. A low number of different genets were also found in two French houses (Maurice et al. submitted). Here they used microsatellite markers to investigate the

population genetic structure in a French population of *S. lacrymans*, and two genets were found in both buildings when multiple isolates were analysed. Thus, even if the sample size in this thesis is rather small, the study by Maurice et al. (submitted) supports the findings of low genotype diversity of *S. lacrymans* within buildings.

For other fungal species a high number of genets have been found to co-occur in relatively close proximity to each other on the same wooden substrate under more natural conditions (Hansen and Hamelin, 1999). For the wood decay fungus *Phellinus nigrolimitatus* a single log of wood harboured at least four genets of the fungus, revealed using VC tests and both ribosomal and single copy nuclear DNA sequences (Kauserud and Schumacher 2002). As many as 19 genets of the pioneer wood decay fungus *Trichaptum abietum* were found to inhabit a single log of Norway spruce (*Picea abies*) in a study using genetic fingerprinting techniques and VC testing (Kauserud and Schumacher 2003). More than one genet was indicated at one of the locations in my study since two MAT genotypes were detected (see below). These were represented by two fruiting bodies that grew in such close proximity that it was difficult to determine by visual inspection whether or not they should be regarded as two separate fruiting bodies or two parts of a larger, irregular fruiting body (Fig. 3b). VC tests assigned all fruiting bodies from this location to the same VC group. This indicates that there are no territorial boundaries between the two genets of the same VC affiliation in *S. lacrymans*. No difference in VC types were detected in any of the locations, so we do not know to which extent two vegetative incompatible genets are capable of sharing a building, or whether or not two such genets would display a higher degree of territoriality than what was observed in this study.

The spatial distribution of a single genet of *S. lacrymans* may be quite extensive. In my study, the fruiting bodies collected from Skåbu where found in every room of the building, and the building in Ås was so thoroughly attacked by the fungus that it was challenging to collect material. Fruiting bodies belonging to one tentative genet were found distributed over at least 40 m^2. Fruiting bodies had also erupted on the outside of the building. It is interesting to note that the ability of single *S. lacrymans* genets to extensively spread and cover a larger area shows similarities to genets of the saprotrofic/necrotrophic *Armillaria* species complex, which are known to cover extremely large territories (Smith et al., 1992). *Armillaria sp.* also resembles *S. lacrymans* in heavy rhizomorph production. But where genets of *Armillaria sp.* in theory

can spread indefinitely through the forest as a connected unit, *S. lacrymans* finds itself limited by the outer walls of a building.

For naturally occurring ectomycorrhizal and saprotrophic species, the number and size of genets occupying an area shows correlations to forest age (Stenlid, 2008). Dahlberg and Stenlid (1994) investigated the spatial distribution of genets in the ectomycorrhizal fungus *Suillus bovinus* by VC tests, and found that the size of genets increased while the number of genets per unit area decreased as the forest aged. Such declines in number of individuals with time are thought to be the result of intraspecific competition, and have also been identified in several other fungal species (Hansen Hamelin 1999 in (Worrall, 1999, Stenlid, 2008). Unfortunately, no time estimates regarding the building infestations in my study are available, making it hard to investigate if such a correlation holds true for *S. lacrymans*.

To date, the local population structure of *S. lacrymans* has not been studied under natural conditions, mainly due to it being found only scarcely in the wild (Palfreyman et al., 2003, Kauserud et al., 2012). In future studies, it could be interesting to compare the population structure of wild *S. lacrymans* to the domesticated ones.

Conclusions

The data presented in this thesis indicate that *S. lacrymans* infections in buildings in Norway often are made up of a single genet, as more than one multi-locus genotype was found in only one location. The results of the VC tests corroborate the findings of earlier studies, stating that vegetative compatibility not necessarily indicate the same genet or individual of *S. lacrymans*, as different genets often display the same VC group affiliation. Spatial distribution of genets could be assessed on one location. Here, two fruiting bodies of different MAT genotype, but sharing the same VC type, partly overlapped each other on the substrate. This suggests a low degree of territoriality among vegetative compatible genets of *S. lacrymans*, probably related to the genets not being able to recognise self from nonself. When assigning MAT genotypes, some sequences did not show the expected polymorphisms. This resulted in the assignment of two identical parental MAT genotypes to some sequences. It seems likely that some variation may be masked due to the selected MAT markers being proxies for the larger MAT complexes, and therefore some molecular variation is likely not reflected by the selected markers, due to the limited resolution. Another possibility relates to the recent discovery of a novel form of homothallism in fungi, referred to as same-sex mating.

However, the genotype distributions revealed by population genetic analyses indicate that outcrossing dominates, so this remains a speculation. Some unexpected polymorphic variation was also found in some sequences from one location, which probably reflects a new MAT B allele not previously described. This was tentatively called b5 in my thesis.

Some caution should be taken when drawing general conclusions. This thesis includes data from six houses only, and for some of the buildings, only a few sequences could be successfully obtained. Therefore, some genotype variation could be masked due to small sample size. Also, how the samples were obtained should be taken into consideration. All the collected samples were fruiting bodies. We have no guarantee that these represent the whole genotype variation found in a building, as mycelia can grow undetected inside wood and walls and cover large parts of a building.

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SUPPLEMENTARY MATERIAL

Table S1: Overview of samples and DNA extraction protocols

Sample ID	Extraction protocol	Date
Odin 6	CTAB with mercaptoethanol	23.01.2012
Odin 7	CTAB with mercaptoethanol	23.01.2012
Odin 7b	CTAB with mercaptoethanol	23.01.2012
Odin 8	CTAB with mercaptoethanol	23.01.2012
Skåbu 1	CTAB with mercaptoethanol	23.01.2012
Skåbu 2	CTAB with mercaptoethanol	23.01.2012
Skåbu 3	CTAB with mercaptoethanol	23.01.2012
Skåbu 5	CTAB with mercaptoethanol	23.01.2012
Skåbu 7	CTAB with mercaptoethanol	23.01.2012
SL 557	CTAB with mercaptoethanol	May 2011
SL 558	CTAB with mercaptoethanol	May 2011
Therese 2	CTAB with mercaptoethanol	21.09.2012
Therese 3	CTAB with mercaptoethanol	21.09.2012
Therese 4	No extraction	-
Therese 5	No extraction	-
Therese T1	Pure CTAB	18.02.2013
Therese T2	Pure CTAB	18.02.2013
Therese T3	Pure CTAB	18.02.2013
Therese T4	Pure CTAB	18.02.2013
Therese T5	Pure CTAB	18.02.2013
Therese T6	Pure CTAB	18.02.2013
Therese T7	Pure CTAB	18.02.2013
Vahl 1	CTAB with mercaptoethanol	25.01.2013
Vahl 2	CTAB with mercaptoethanol	25.01.2013
Vahl 3	Pure CTAB	16.02.2013
Vahl 8	Pure CTAB	16.02.2013
Ås 1	Pure CTAB	16.02.2013
Ås 2	Pure CTAB	16.02.2013
Ås 3	CTAB with mercaptoethanol	21.09.2012
Ås 5	Pure CTAB	16.02.2013
Ås 8	Pure CTAB	16.02.2013
Ås 9	Pure CTAB	16.02.2013

Ås 10	Pure CTAB	16.02.2013
Ås 12	Pure CTAB	16.02.2013
Ås 13	Pure CTAB	16.02.2013
Ås 14	Pure CTAB	16.02.2013
Ås 15	Pure CTAB	16.02.2013
Ås 16	Pure CTAB	16.02.2013

Table S2: Successful amplifications

Y indicates that a sequence was obtained, while N indicates that no sequence was obtained.

Sequence ID	MAT A	MAT B	Sequence ID	MAT A	MAT B
Therese T1	Y	Y	Ås 1	Y	Y
Therese T2	Y	Y	Ås 2	N	Y
Therese T4	Y	N	Ås 3	Y	N
Therese T5	Y	N	Ås 4	Y	N
Therese T6	Y	N	Ås 5	Y	Y
Therese T7	Y	Y	Ås 6	Y	N
Therese 2	Y	N	Ås 7	Y	N
Therese 3	Y	Y	Ås 8	Y	Y
Vahl 1	Y	N	Ås 9	N	Y
Vahl 2	Y	N	Ås 10	N	Y
Vahl 3	Y	Y	Ås 12	N	Y
Vahl 8	Y	N	Ås 13	N	Y
Skåbu 1	Y	Y	Ås 14	N	Y
Skåbu 2	Y	Y	Ås 15	N	Y
Skåbu 3	Y	Y	Ås 16	N	Y
Skåbu 4	N	Y	Odin 6	Y	Y
Skåbu 5	Y	N	Odin 7	Y	Y
Skåbu 7	Y	Y	Odin 7b	Y	Y
SL 557	Y	N	Odin 8	Y	Y
SL558	Y	Y			

Fig. S1: Alignment of MAT A sequences

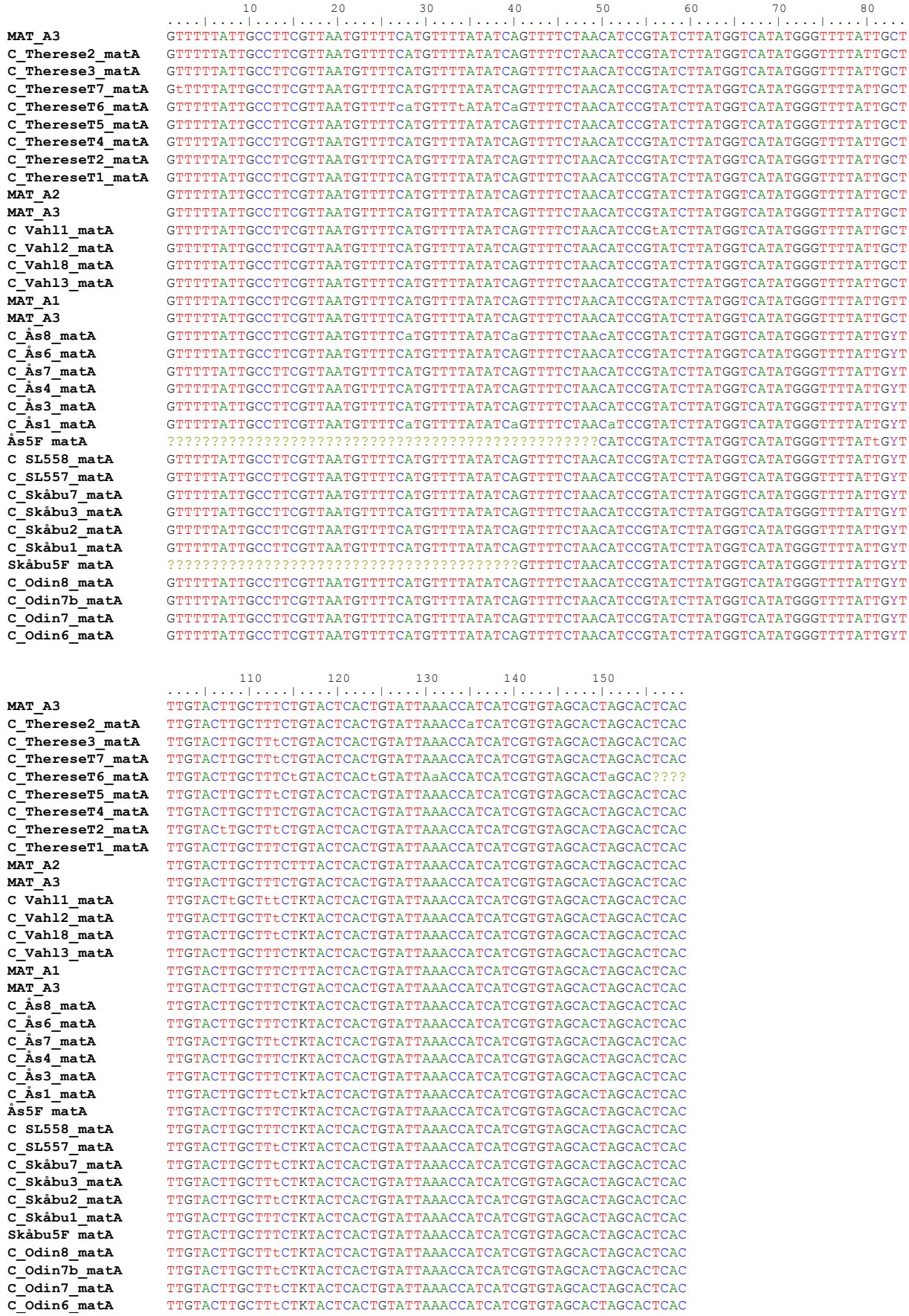
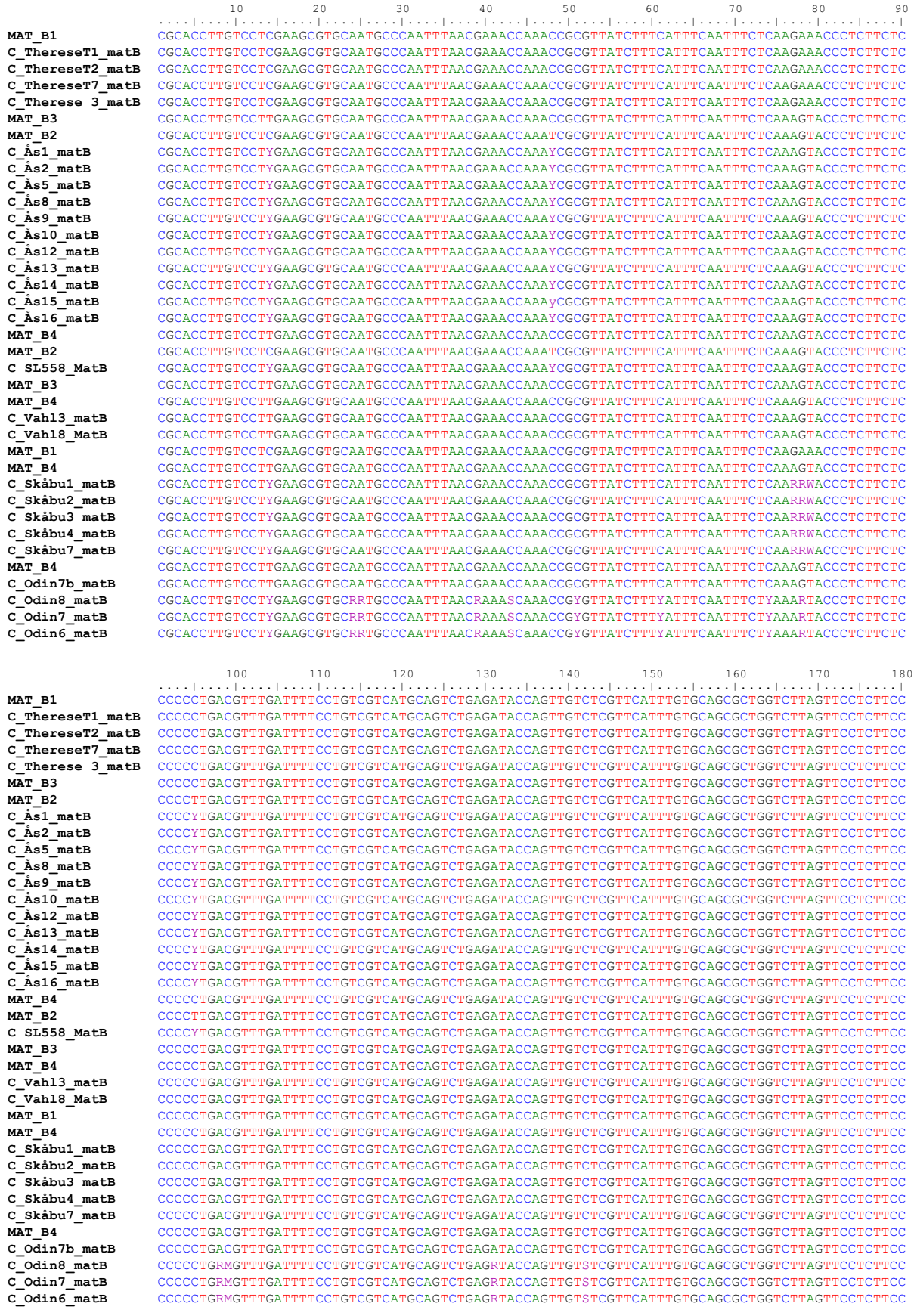


Fig. S2: Alignment of MAT B sequences

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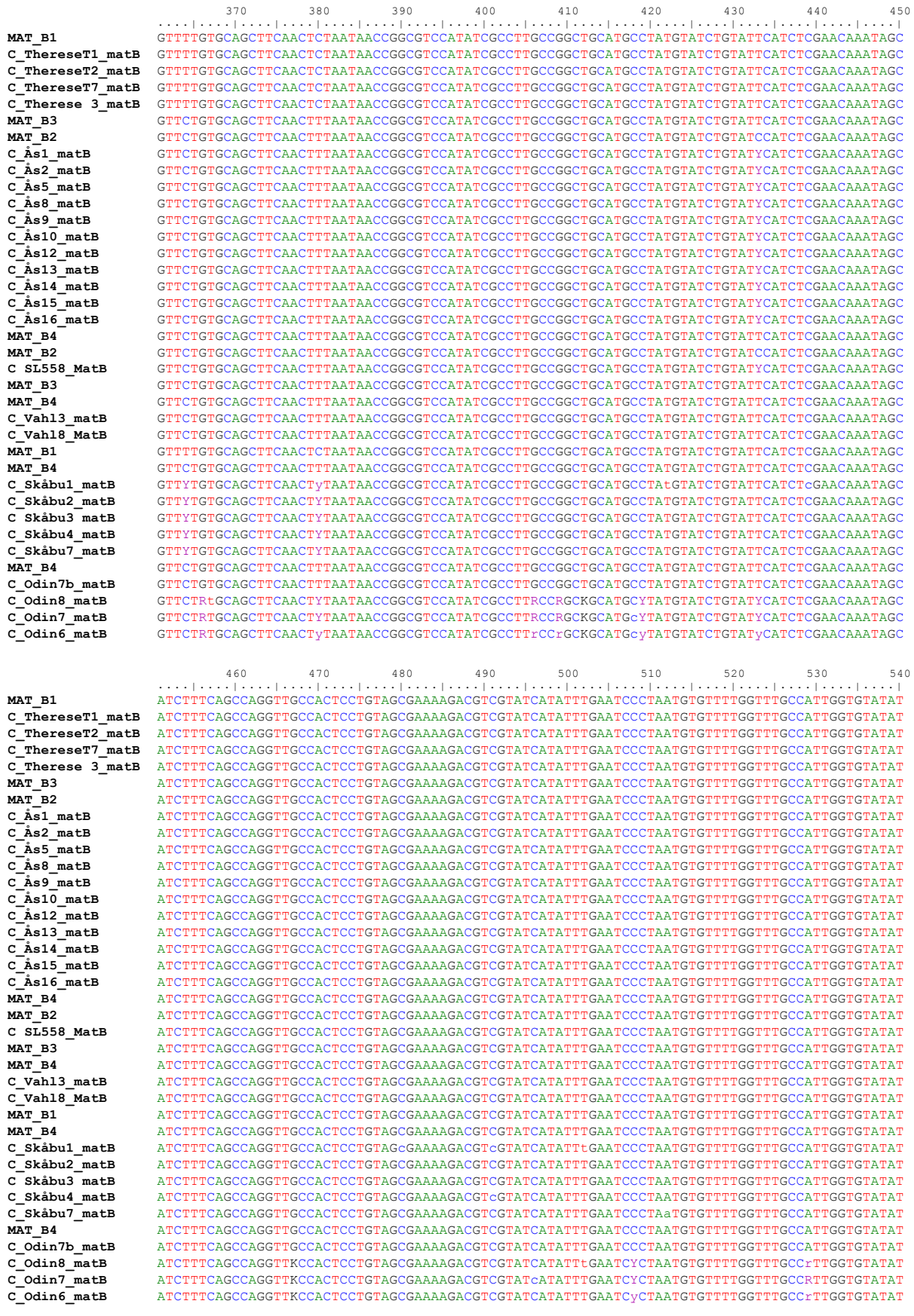
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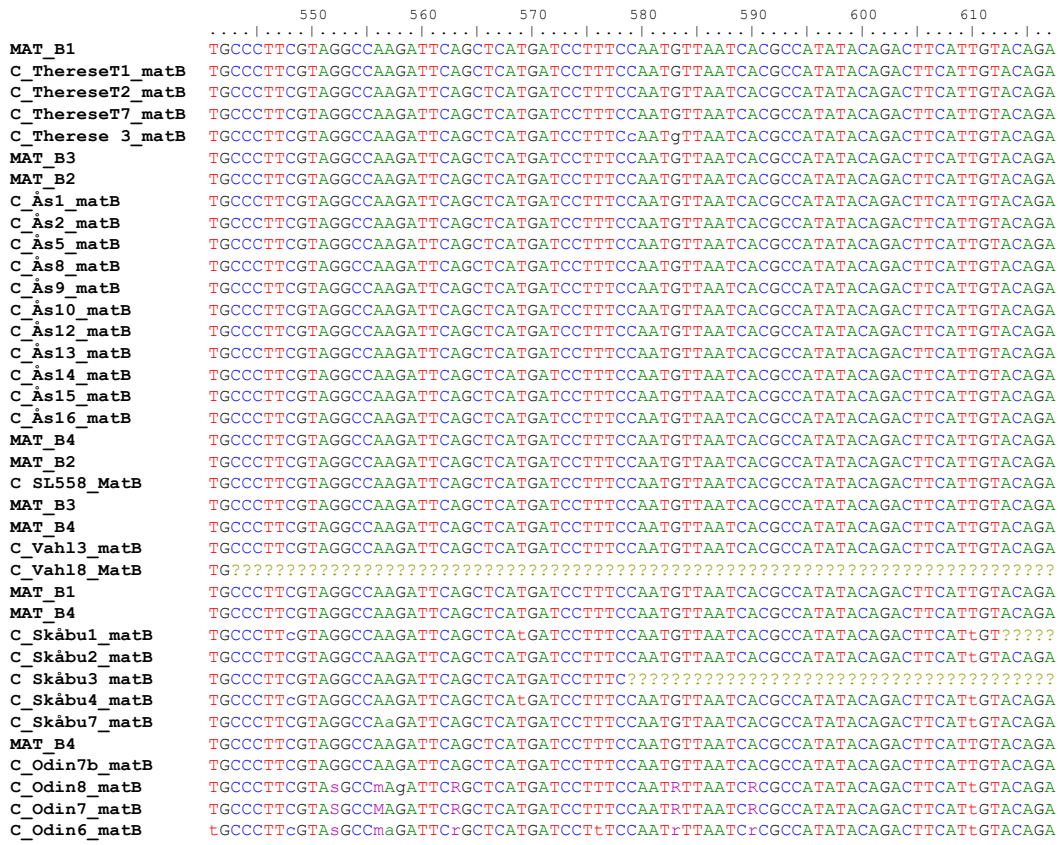
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C_ThereseT2_matB TGCCCTTCGTAGGCCAAGATTCAGCTCATGATCCTTTCCAATGTTAATCACGCCATATACAGACTTCATTGTACAGA
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C_Ås1_matB TGCCCTTCGTAGGCCAAGATTCAGCTCATGATCCTTTCCAATGTTAATCACGCCATATACAGACTTCATTGTACAGA
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Fig. S3: Picture from Odins gate.